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Modified Sperm Ultrastructure in Four Species of Soft-Bodied Echinoids (Echinodermata: Echinothuriidae) From the Bathyal Zone of the Deep Sea

KEVIN J. ECKELBARGER, CRAIG M. YOUNG, AND J. LANE CAMERON

Division of Marine Sciences, Harbor Branch Oceanographic Institution, 5600 Old Dixie Highway, Fort Pierce, Florida 34946

Abstract. Sperm of the bathyal echinothuriid echinoids *Phormosoma placenta, Sperosoma antillense, Araeosoma fenestratum,* and *A. belli* are similar to those of other echinoids, but have several unique morphological features involving the acrosomal vesicle, the nucleus, and the middlepiece. The acrosomal vesicle shows regional staining differences including a densely staining central region surrounded by an electron-opaque component. Sperm nuclei are highly elongated and abruptly taper posteriorly. With the exception of one species, the nuclei lack a distinct centriolar fossa. Intracellular droplets resembling lipid extend from the extreme posterior region of the middlepiece to form a collar around the proximal portion of the axoneme. The presence of lipid-like bodies in the middlepiece suggest that the sperm are long-lived and therefore require additional energy stores not found in most metazoan sperm. These findings are compared with a similar study of sperm ultrastructure in three shallow-water echinothuriid species, and their potential significance is discussed in relation to the present knowledge of echinothuriid reproductive biology.

Introduction

Echinothuriid echinoids have soft, flexible tests atypical of most sea urchins. The majority of species are confined to the deep sea but some littoral species are reported from the Indo-Pacific region (Amemiya *et al.,* 1980). Our knowledge of the reproductive biology of members of this family has been limited mostly to general observations of a few deep-sea species (Mortensen, 1927; Ahfeld, 1977). Tyler and Gage (1984) have examined gametogenesis in five echinothuriids from the Rockall Trough in the Northeast Atlantic Ocean using light microscopic histology. They suggest that the dominant reproductive strategy is the year-round production of large eggs undergoing benthic direct development. However, other authors have reported that echinothuriid eggs float (Amemiya and Tsuchiya, 1979; Young and Cameron, 1987; Cameron *et al.,* 1988) suggesting that the larvae are probably pelagic lecithotrophs.

Echinoderms are among the most abundant and diverse macrofaunal invertebrates in the deep sea (Pawson, 1982; Billet and Hansen, 1982), yet little is known about gamete and gonad ultrastructure in species from this habitat. The only published account deals with the ultrastructure of the highly aberrant sperm of the deep-sea concentricycloid *Xyloplax* (Healy *et al.,* 1988). General sperm morphology is known for nearly 70 shallow-water echinoids, although fewer than 10 species have been the subject of ultrastructural studies. These combined investigations show that echinoid sperm are consistently conservative in morphology and exhibit little interspecific structural variation (see Summers *et al.,* 1975; Chia and Bickell, 1983, for review).

The present paper describes ultrastructural features of the mature sperm of the bathyal echinothuriids *Phormosoma placenta, Sperosoma antillense, Araeosoma fenestratum,* and *A. belli* collected in the Bahama Islands by manned submersibles at depths ranging from 600 to 900 meters. Although their sperm are similar to those of other echinoids, significant ultrastructural differences are noted in the acrosome, nucleus, and middlepiece. Sperm morphology is influenced by the environment into which they are released prior to fertilization (Franzen, 1956, 1970; Afzelius, 1977), so structural alterations in these sperm suggest that unique selective pressures may be present in the deep sea which are absent from shallow water habitats.

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These results are compared with an earlier ultrastructural study of sperm morphology in three species of shallow-water echinothuriids from Japanese waters (Amemiya *et al.*, 1980). The potential significance of these findings are discussed in relation to echinothuriid reproductive biology.

Materials and Methods

Live, sexually mature specimens of *Phormosoma placenta* (Fig. 1), *Sperosoma antillense*, *Araeosoma fenestratum* (Fig. 2) and *A. belli* were collected during April, May, and October, 1986, at depths ranging from 600 to 900 m in the Bahamas. The seawater temperature at the various collection sites ranged from 7 to 10°C. Collection sites included the west side of San Salvador Is. (24°02′N, 74°32.4′W), the north side of New Providence Is. (25°03.1′N, 77°31.4′W), Southwest Reef (24°53.41′N, 77°33.14′W), and the east side of Andros Is. (24°51.4′N, 77°50.62′W). Specimens were obtained using specialized collecting equipment on Johnson-Sea-Link submersibles.

In most instances, live animals were dissected on board ship shortly after collection, and testes were removed for immediate fixation. Occasionally, however, specimens were maintained on board in cooled aquaria where they remained active and healthy until later use. Whole testes were fixed for transmission electron microscopy (TEM); several methods, including those of Eckelbarger (1979) and Smiley (1988), were used, although none produced satisfactory results. Acceptable results were finally obtained with a modification of the method published by Bickell *et al.* (1980). Tissue was fixed by immersion for 1 h in a primary fixative consisting of 2.5% glutaraldehyde in filtered seawater at room temperature, followed by a 15-min wash in 2.5% $NaHCO_3$, and postfixation for 1 h in 2% OsO_4 in 1.25% $NaHCO_3$ at room temperature. Tissues were rapidly dehydrated in ascending concentrations of ethanol, transferred through two changes of propylene oxide, and embedded in Epon. Thin sections were cut with a diamond knife and stained with alcoholic uranyl acetate and aqueous lead citrate for 10 min each, then examined with a Zeiss EM9S-2 transmission electron microscope.

Live active sperm released from dissected testes were collected and fixed for scanning electron microscopy (SEM). Sperm were fixed using the same procedure as for TEM, followed by dehydration through an ascending ethanol series to 50%, air-dried on cover slips attached to aluminum stubs, and sputter-coated with gold-palladium. Sperm were then photographed using a Novascan 30 SEM with an accelerating voltage of 15 kV.

Results

The mature spermatozoa of all four species are similar in general morphology but differ in head length (acrosome, nucleus, and middlepiece) and relative dimensions. They are bullet-shaped with a small, terminal acrosome, a conical nucleus, and short middlepiece (Figs. 5–12). The sperm head length of the four species range from the longest, *Phormosoma placenta* (12 × 1.5 μm), to the shortest, *Araeosoma fenestratum* (8.5 × 1.0 μm). The sperm heads of *A. belli* and *Sperosoma antillense* each measure 9.0 × 1.25 μm. All four species have large, yolky eggs, ranging from the largest, *Araeosoma fenestratum* (1290 μm), to *Sperosoma antillense* (1060 μm), *Araeosoma belli* (965 μm), and the smallest, *Phormosoma placenta* (890 μm).

The acrosome is positioned just anterior to the nucleus and consists of a subspherical acrosomal vesicle with a flattened or slightly concave basal surface (Figs. 3, 4). The acrosomal membrane and the sperm plasmalemma are not well defined due to poor fixation. However, the posterior-lateral acrosomal membrane is thicker and more electron dense than the apical portion. The acrosomal vesicle contains two types of granular material of differing electron densities. The central portion contains a more densely staining vase-shaped region surrounded laterally by an electron-opaque component. A deep subacrosomal fossa is present just posterior to the acrosomal vesicle. Although the acrosomal region of all four species are similar, the subacrosomal fossa of *Sperosoma antillense* (Fig. 4) is narrower and deeper than that of the other species, typified by *A. belli* (Fig. 3). The subacrosomal fossa contains granular periacrosomal material extending around the basolateral sides of the acrosomal vesicles and running posteriorly between the anterolateral margin of the nucleus and sperm plasmalemma.

The nucleus contains highly condensed chromatin and gradually tapers anteriorly in all four species. Nuclear vacuoles are common in all sperm examined (Fig. 15). In the sperm of *Phormosoma placenta*, the extreme posterior region of the nucleus narrows abruptly to form a neck-like extension that extends into the middle piece (Figs. 9, 13). This posterior extension tapers more gradually in the sperm of *Sperosoma antillense* (Figs. 10, 14), *Araeosoma fenestratum* (Figs. 11, 15), and *A. belli* (Figs. 12, 16). The posterior surface of this extension is slightly concave and the axoneme-bearing distal centriole is closely associated with it (Figs. 13–16). A distinct centriolar fossa is absent except for a shallow one in *P. placenta* (Fig. 13). The proximal centriole is positioned laterally to the distal centriole and at a slight angle to the long axis of the sperm (Figs. 15, 16, 18). The distal centriole possesses a centriolar satellite consisting of nine radiating arms (Fig. 19). A single, circular mitochondrion surrounds the posterior nuclear extension (Fig. 17). In the sperm of all four species, intracellular droplets morphologically resembling lipid, extend from the extreme posterior region of the middlepiece (Figs. 13–16). They are consistently round in *P. placenta* (Fig. 13), but

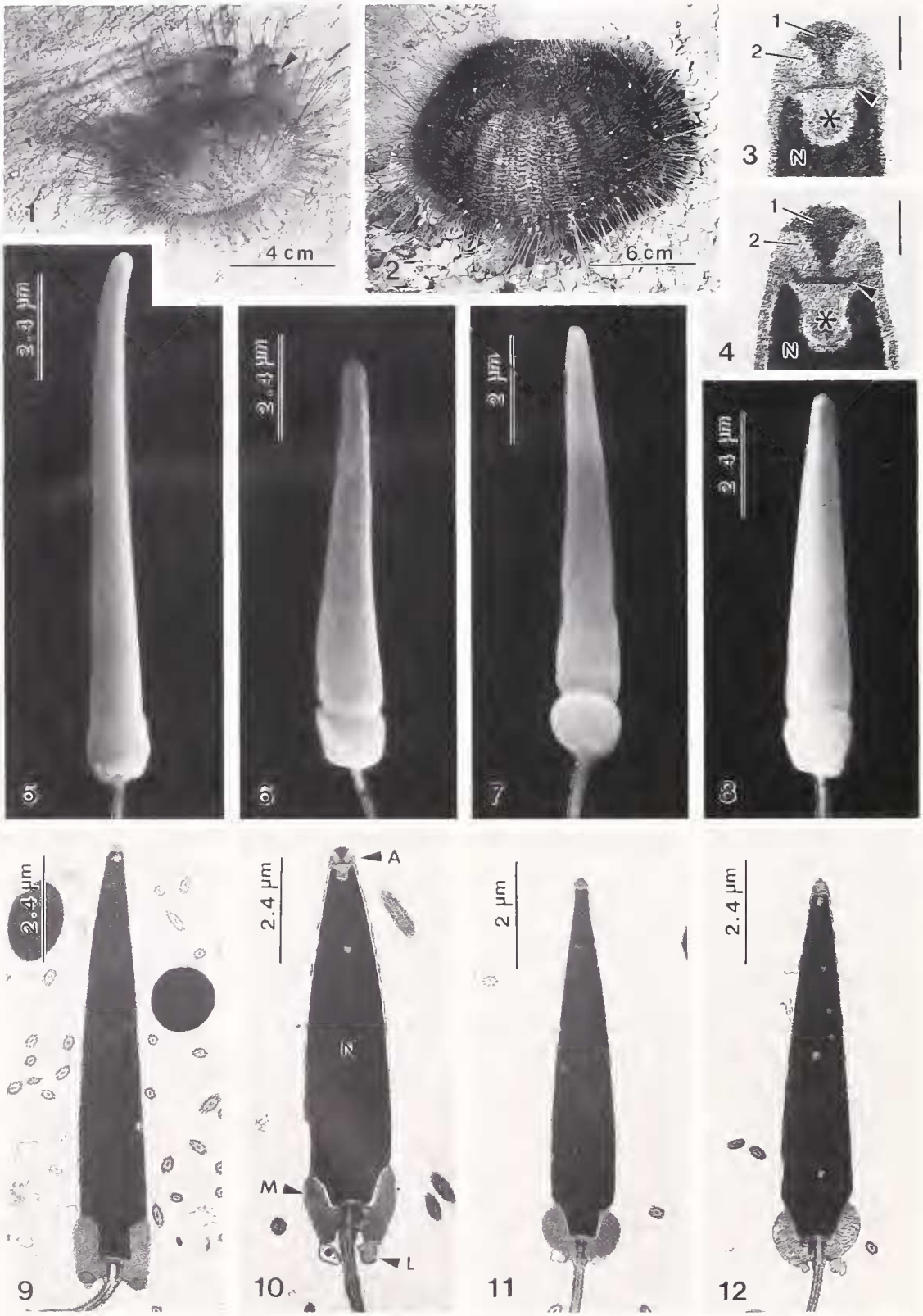


Figure 1. Adult *Phormosoma placenta* photographed *in situ* at a depth of 700 m in Bahamian waters. Arrow indicates one of several epidermal sacs of unknown function which project from the surface of the animal.

Figure 2. Adult *Araeosoma belli* photographed *in situ* at a depth of 750 m in Bahamian waters.

irregular in shape in the other species (Figs. 14–16). In cross section, a collar of lipid-like droplets surrounds the basal region of the axoneme (Fig. 20). The axoneme is of the 9 + 2 pattern.

Discussion

Recent investigations of sperm morphology from deep-sea echinoderms have revealed a variety of structural modifications that differ markedly from the sperm of shallow water species. These include a high incidence of elongated sperm heads in bathyal echinoids and holothuroids (Eckelbarger *et al.*, in press), the discovery of dimorphic sperm in the abyssal echinoid *Phrissocystis multispina* (Eckelbarger *et al.*, 1989), and the presence of highly aberrant sperm in the abyssal concentricycloid *Xyloplax turnerae* (Healy *et al.*, 1988). The present paper describes additional modifications of male gametes from four species of bathyal echinoids from Bahamian waters.

The sperm acrosomes of echinoids are morphologically conservative and have been described ultrastructurally for a number of species (see Chia and Bickell, 1983). The acrosomal vesicle typically contains a homogeneously distributed particulate material of medium electron density except for its basal region where denser material is deposited along the inner acrosomal membrane. The sperm acrosomes of all four echinothuriid species we examined resemble those of other echinoids with respect to position and superficial morphology, but show unique regional staining differences within the acrosomal vesicle. In other echinoderm classes, the contents of the acrosomal vesicle are often homogeneous, although densely staining, centrally positioned acrosomal granules have been reported in the holothuroids *Cucumaria lubrica, C. miniata,* and *Leptosynapta clarki* (Atwood and Chia, 1974), and the crinoid, *Antedon petasus* (Afzelius, 1977). Therefore, the echinothuriid acrosomal vesicle represents a morphological variant different from any observed in other echinoderm sperm. We do not believe this structural variation results from a fixation artifact because no differences were observed in sperm prepared for electron microscopy using several fixation methods. The functional significance of this novel acrosomal morphology is unknown, but it may reflect regional differences in enzyme distribution such as that observed in the cortical granules of sea urchin eggs (Alliegro and Schuel, 1988).

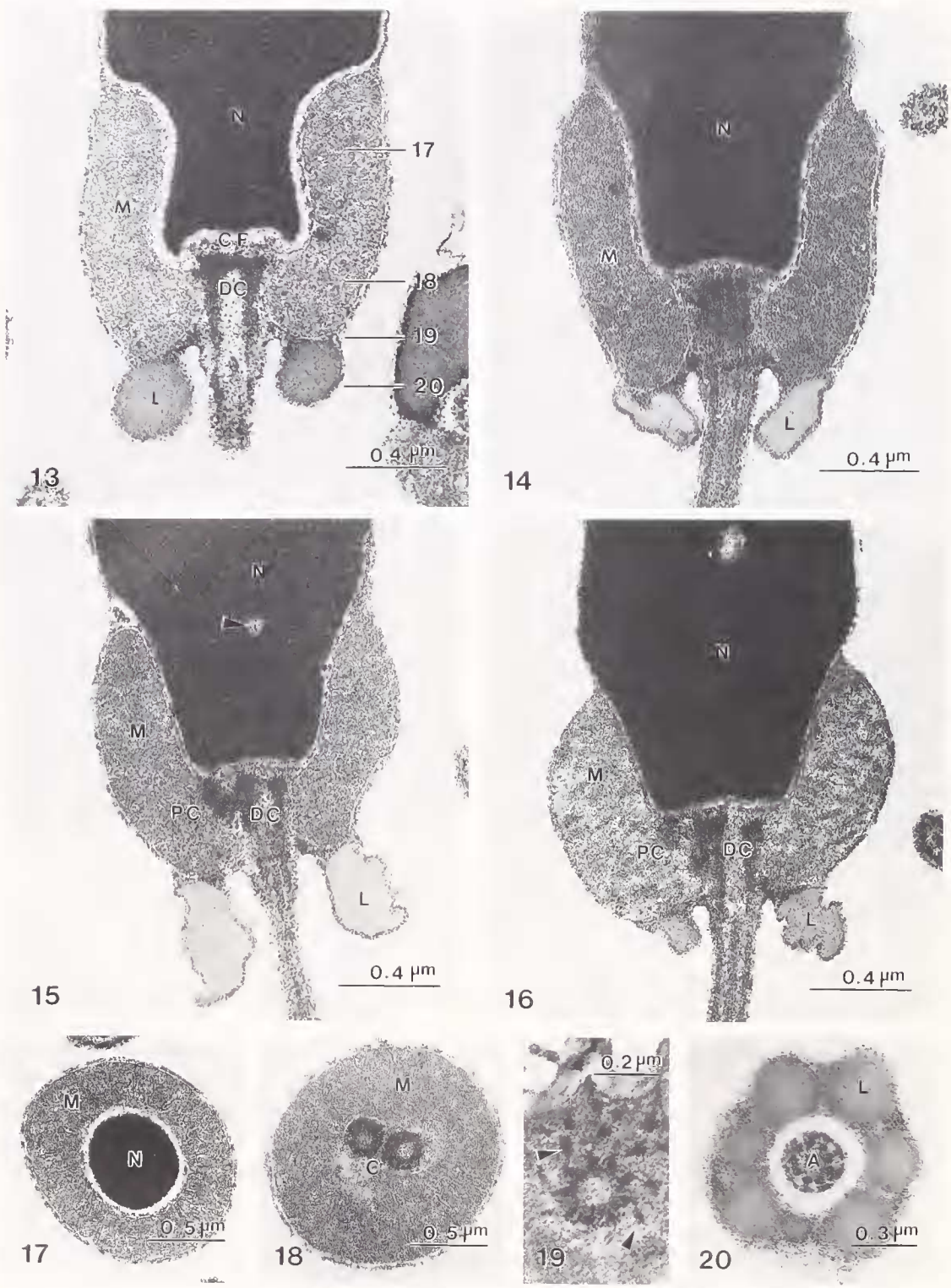
Echinothuriid sperm nuclei have a shape unique to echinoids. Echinoid sperm nuclei typically are wider posteriorly than anteriorly and have a relatively deep centriolar fossa into which the distal centriole and its associated axoneme are inserted (Chia and Bickell, 1983). Echinothuriid sperm nuclei abruptly taper posteriorly and a centriolar fossa is virtually absent in *Sperosoma, Araeosoma belli,* and *A. fenestratum,* and weakly developed in *Phoromosa placenta.* The presence of a deep centriolar fossa in echinoderm sperm has been suggested as a means of strengthening the connection between the tail and the elongated nucleus (Chia and Bickell, 1983). The position of the mitochondrion relative to the nucleus varies slightly in echinoderm sperm, but generally surrounds the extreme posterior end of the nucleus (Chia and Bickell, 1983). In some echinoids, such as *Paracentrotus lividus* (Anderson, 1968), *Strongylocentrotus purpuratus* (Longo and Anderson, 1969), and *Arbacia lixula* and *Echinometra lucunter* (Cruz-Landim and Beig, 1976), the mitochondrion is positioned posterior to the nucleus and forms a collar around the proximal portion of the axoneme. In the four echinothuriid species, the sperm mitochondrion wraps around the posterior portion of the nucleus, starting where the nucleus abruptly tapers. The modified sperm of the small brooding holothuroid *Cucumaria lubrica* is strikingly similar to the echinothuriid sperm with respect to nuclear shape and mitochondrial position (Atwood and Chia, 1974), although a well-developed centriolar fossa is present in *C. lubrica.*

The lipid-like droplets we observed in the middlepiece of the echinothuriid sperm closely resemble intracellular triglyceride deposits that are common in a variety of somatic cells (Bloom and Fawcett, 1968; Alberts *et al.*, 1983). To our knowledge, there are no other reports of lipid deposits in metazoan sperm aside from a few echinoderm species. Intracellular deposits that resemble lipid on morphological grounds, have been reported posterior to the middlepiece mitochondrion in the echinoids *Echinorachnius parma* (Summers and Hylander, 1974; Summers *et al.*, 1975), *Arbacia punctulata* (Bernstein, 1962; Longo and Anderson, 1969), *Echinocardium flavescens*

Figures 3, 4. Anterior region of mature sperm of *Araeosoma belli* (Fig. 3) and *Sperosoma antillense* (Fig. 4) showing acrosomal vesicle with two regions of differing electron densities. Arrow indicates dense staining posterior acrosomal membrane; *, subacrosomal fossa; N, nucleus.

Figures 5–8. Scanning electron micrographs of mature sperm of *Phormosoma placenta* (Fig. 5), *Sperosoma antillense* (Fig. 6), *Araeosoma fenestratum* (Fig. 7), and *A. belli* (Fig. 8).

Figures 9–12. Transmission electron micrographs of longitudinal sections through mature sperm of *Phormosoma placenta* (Fig. 9), *Sperosoma antillense* (Fig. 10), *Araeosoma fenestratum* (Fig. 11), and *A. belli* (Fig. 12). The sperm acrosomes in Figures 9, 11, and 12 are not clearly indicated due to the slightly oblique angle of the sections. A, acrosome; N, nucleus; M, mitochondrion; L, lipid-like deposit.



Figures 13–16. Longitudinal sections through the posterior region of the mature sperm of *Phormosoma placenta* (Fig. 13), *Sperosoma antillense* (Fig. 14), *Aracosoma fenestratum* (Fig. 15), and *A. belli* (Fig. 16). Horizontal lines in Fig. 13 indicate level of cross-sections through sperm of *Phormosoma placenta* shown in Figures 17–20. N, nucleus; M, mitochondrion; CF, centriolar fossa; DC, distal centriole; PC, proximal centriole; L, lipid-like deposit.

Figure 17. Cross section through posterior nucleus (N) and surrounding mitochondrion (M) of sperm of *P. placenta*.

and *Brissopsis lyrifera* (Afzelius and Mohri, 1966), *Hapalosoma gemmiferum* and *Araeosoma owstoni* (Amemiya *et al.*, 1980), and in the holothuroid *Cucumaria miniata* (Fontaine and Lambert, 1976). However, qualitative comparisons of micrographs from the above studies show that, with the exception of *E. parma* (Summers and Hylander, 1974) and *A. owstoni* (Amemiya *et al.*, 1980), these deposits are minor in comparison to those observed in the present study. The role of lipid deposits has not been determined in any of these echinoderm sperm.

Echinoderm spermatozoa are dependent on the metabolism of mitochondrial phospholipids during swimming (Rothschild and Cleland, 1952), or in some instances, glycogen stores (Anderson and Personne, 1975). In experiments with the sperm of the echinoid *Brissopsis lyrifera*, Afzelius and Mohri (1966) reported that prolonged swimming caused a gradual disappearance of mitochondrial cristae. They hypothesized that the sperm consume these structural phospholipids as an energy source. However, no change was observed in the lipid-like inclusions after 6 h of swimming. Triglycerides of fatty acids are used as an energy reserve, and they are commonly associated with mitochondria (Alberts *et al.*, 1983). The presence of lipid deposits in intimate association with mitochondria in some echinoderm sperm suggests that the cells are long-lived or must expend energy at a rate not commonly demanded of other metazoan sperm. We have not observed spawning in any of the echinothuriids we examined, and we know nothing of their fertilization biology or the potential life span of naturally released sperm. With the occasional exception of *Phormosoma placenta*, adult echinothuriids generally occur at very low densities in the Bahamas. The extensive lipid stores in these sperm could provide an extended window of opportunity for fertilization when males and females are widely separated. Some cidarid urchins improve fertilization success by aggregating during the breeding season (Young, unpub. data). However, echinothuriids appear not to breed seasonally (Tyler and Gage, 1984) nor to aggregate for spawning.

The present study of bathyal echinothuriid sperm morphology provides an interesting comparison to an earlier investigation of sperm ultrastructure in the three shallow-water echinothuriids *Asthenosoma ijimai*, *Araeosoma owstoni*, and *Hapalosoma gemmiferum* (Amemiya *et al.*, 1980). The latter authors described sperm morphologies very similar to those in the present study, including regional staining differences within the acrosomal vesicles, a long, tapering nucleus that abruptly narrows posteriorly, and "follicular bodies" in the middlepiece of *H. gemmiferum* and *A. owstoni* sperm, which they viewed as analagous to the lipid-like bodies reported from other echinoid sperm. However, they reported an "electron opaque rod" within the acrosomal vesicle. In addition, the lipid-like droplets appear to be smaller than those we described, and they do not extend from the posterior region of the middlepiece as they do in the deep-sea echinothuriid sperm. No information was presented regarding the process of natural spawning or sperm longevity. The most striking difference noted between our two studies is the shorter head lengths in the sperm of the shallow-water species. Sperm head length was estimated to be 7 μm for *A. ijimai*, 6 μm for *A. owstoni*, and 4 μm for *H. gemmiferum*. In contrast, we measured sperm head lengths of 12 μm for *Phormosoma placenta*, 8.5 μm for *A. fenestratum*, and 9 μm for *A. belli* and *Sperosoma antillense*. A high incidence of elongated sperm heads was noted recently in a survey of bathyal echinoids (Eckelbarger *et al.*, in press).

Ultrastructural studies of the gametes of deep-sea organisms have been rare, with only three studies of gamete morphology and sperm development in the vestimintiferan *Riftia pachyptila* (Gardiner and Jones, 1985; Cary *et al.*, 1989), and spermiogenesis in the abyssal echinoderm *Xyloplax turnerae* (Healy *et al.*, 1988) having been published recently. This is unfortunate because deep-sea habitats offer a unique laboratory for the study of gamete evolution in an environment substantially different from that of shelf waters (see Wilson and Hessler, 1987). Indeed, recent observations of unique gonadal, gamete, and larval developmental features in bathyal and abyssal echinoderms have demonstrated that deep-sea echinoderms have undergone evolutionary changes in their reproductive biology unlike that of their shallow water relatives (Eckelbarger *et al.*, in press, 1989; Young *et al.*, in press). This suggests that the deep sea is a region ripe for studies dealing with the reproductive evolution of marine invertebrates.

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Figure 18. Cross section through centrioles (C) and surrounding mitochondrion (M) in middlepiece of *P. placenta* sperm.

Figure 19. Cross section through centriolar satellite showing nine radiating arms (arrows) in middlepiece of sperm of *P. placenta*.

Figure 20. Cross section through middlepiece of sperm of *P. placenta* showing ring of lipid-like bodies (L) surrounding proximal portion of axoneme (A).

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